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## BBA Report

BBA 20056

### A SIMPLE PROCEDURE FOR THE SYNTHESIS OF [ $^{32}\text{P}$ ]PHOSPHOENOLPYRUVATE VIA THE PYRUVATE KINASE EXCHANGE REACTION AT EQUILIBRIUM

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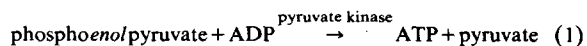
(Received June 6th, 1983)

**Key words:** Phosphoenolpyruvate synthesis; Pyruvate kinase; Phosphate transfer

A one step procedure is presented for the preparation of [ $^{32}\text{P}$ ]phosphoenolpyruvate from [ $\gamma$ - $^{32}\text{P}$ ]ATP using pyruvate kinase. The reaction is carried out at chemical equilibrium and involves only an exchange of isotope between ATP and phosphoenolpyruvate. The initial phosphoenolpyruvate/ATP ratio in the reaction mixture determines the degree of  $^{32}\text{P}$  incorporation into phosphoenolpyruvate when isotopic equilibrium is achieved.

[ $^{32}\text{P}$ ]phosphoenolpyruvate is an essential reagent in biosynthetic studies and enzyme mechanistic studies involving phosphoryl group transfer from this substrate. It is not commercially available but there are three procedures for its preparation. The oldest procedure [1] is a chemical synthesis starting from  $\beta$ -chlorolactic acid and  $^{32}\text{P}$  inorganic phosphate. We have had only limited success with this method. In the last year two procedures utilizing phosphoenolpyruvate carboxykinase have been published [2,3]. The procedure of Parra [2] uses the GTP-dependent enzyme from liver mitochondria and the procedure of Mattoo and Waygood [3] makes use of the *E. coli* ATP-dependent enzyme. Neither enzyme is commercially available, thus at least a partial enzyme extraction must be done prior to the synthesis.

The procedure presented in this report describes the synthesis of [ $^{32}\text{P}$ ]phosphoenolpyruvate from [ $\gamma$ - $^{32}\text{P}$ ]ATP using a commercially available enzyme, pyruvate kinase. The equilibrium of the pyruvate kinase catalyzed reaction (Eqn. 1) lies far to the right ( $\Delta G^{\circ} = -5.7$  kcal/mol).



Consequently, a straightforward synthesis from [ $\gamma$ - $^{32}\text{P}$ ]ATP and pyruvate would necessitate a prohibitively large excess of [ $\gamma$ - $^{32}\text{P}$ ]ATP and pyruvate to produce only a small amount of [ $^{32}\text{P}$ ]phosphoenolpyruvate. However, when pyruvate kinase is incubated with [ $\gamma$ - $^{32}\text{P}$ ]ATP, phosphoenolpyruvate and pyruvate but without ADP, a small amount of ADP and [ $^{32}\text{P}$ ]phosphoenolpyruvate will be produced from ATP in order to establish chemical equilibrium. Isotopic equilibrium will then be achieved by the interconversion of ATP and phosphoenolpyruvate without any further change in the chemical concentrations of the two substrates. At isotopic equilibrium the ratio of  $^{32}\text{P}$  label in the phosphoenolpyruvate and ATP pools will be determined only by their initial concentrations.

A 1 ml reaction mixture was prepared containing the following components: 0.1 M triethylamine pH 7.6, 3 mM  $\text{MgCl}_2$ , 15 mM KCl, 1 mM pyruvate, 100  $\mu\text{M}$  phosphoenolpyruvate (cyclohexylammonium salt), 10  $\mu\text{M}$  [ $\gamma$ - $^{32}\text{P}$ ]ATP ( $2 \cdot 10^4$  Ci/mol) and 40 units of pyruvate kinase. The mixture was incubated for 90 min at 30°C after which it was diluted ten times with water and chromatographed on a (100  $\times$  7 mm) column of Dowex AG-1-X8 in triethyl ammonium bi-

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carbonate following the procedure of Mattoo and Waygood [3]. The chromatography resulted in a 10 ml pool containing  $9.5 \mu\text{M}$  [ $^{32}\text{P}$ ]phosphoenolpyruvate ( $1.8 \cdot 10^3 \text{ Ci/mol}$ ). The triethyl ammonium bicarbonate can be removed by rotary evaporation if desired. When the reaction is allowed to go to isotopic equilibrium the specific activity of the [ $^{32}\text{P}$ ]phosphoenolpyruvate can be calculated from the known specific activity of the ATP and the initial ATP and phosphoenolpyruvate concentrations. A separate determination of the concentration is unnecessary. In order to show

that the procedure actually yields the expected amount of phosphoenolpyruvate, we have carried out an independent analysis. The phosphoenolpyruvate concentration of the purified product was determined enzymatically by converting it quantitatively to [ $^{14}\text{C}$ ]mannitol 1-phosphate using purified components of the phosphoenolpyruvate-dependent mannitol phosphotransferase system. Approximately  $5 \mu\text{M}$  phosphoenolpyruvate was incubated at  $37^\circ\text{C}$  in a reaction mixture containing purified phospho carrier (HPr; Ref. 4), enzyme I (EI; Ref. 5), enzyme II<sup>mtl</sup> (EII<sup>mtl</sup>; Ref. 6),  $20 \mu\text{M}$  [ $^{14}\text{C}$ ]mannitol, 25 mM Tris-HCl buffer (pH 7.8), 5 mM  $\text{MgCl}_2$ , 10 mM NaF, 5 mM dithiothreitol and 0.03% Lubrol PX. The initial phosphoenolpyruvate concentration in such an assay is equal to the final [ $^{14}\text{C}$ ]mannitol 1-phosphate concentration reached upon completion of the reaction.

Fig. 1A shows the progress of an exchange reaction as a function of time. In this particular reaction containing only 20 units of pyruvate kinase, equilibrium was reached after approx. 150 min. No product other than [ $^{32}\text{P}$ ]phosphoenolpyruvate appeared during the incubation period. Fig. 1B presents a chromatogram of the reaction mixture and the purified product prepared as described above. In order to determine whether the product obtained from the Dowex chromatography was [ $^{32}\text{P}$ ]phosphoenolpyruvate, an aliquot of the chromatographed material was treated with pyruvate kinase and ADP. All of the material was converted back to a product chromatographing with the same  $R_F$  as [ $\gamma\text{-}^{32}\text{P}$ ]ATP.

These data show that [ $^{32}\text{P}$ ]phosphoenolpyruvate can be synthesized in a one step reaction from [ $\gamma\text{-}^{32}\text{P}$ ]ATP via the pyruvate kinase catalyzed exchange reaction at equilibrium. The advantage of this procedure is that it can be done with a commercially available enzyme preparation. The extent of the incorporation of  $^{32}\text{P}$  label into phosphoenolpyruvate is determined only by the ratio of the phosphoenolpyruvate and ATP concentrations. This method of [ $^{32}\text{P}$ ]phosphoenolpyruvate synthesis results in a preparation with a decreased specific activity relative to the starting material. Considering the high specific activities of commercially available ATP, this is not a significant problem.

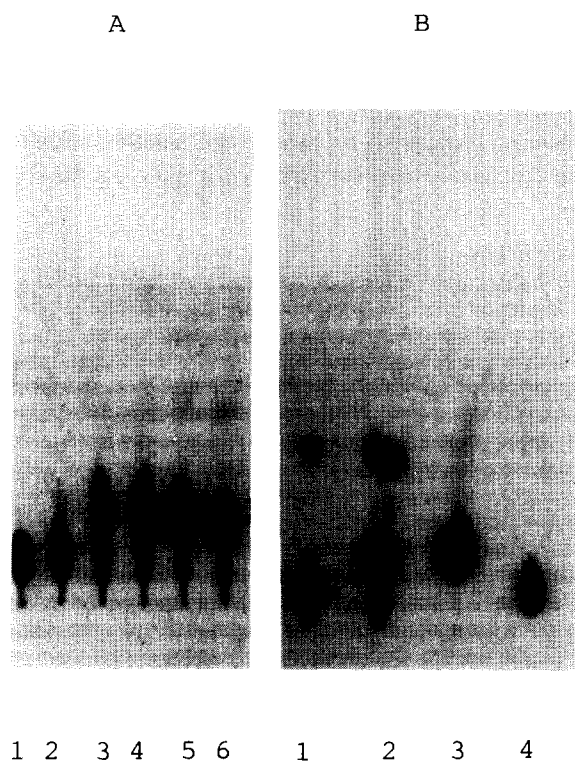


Fig. 1. Autoradiograms of polyethyleneimine thin layer chromatograms developed in 2 M sodium formate buffer, pH 3.4 [2]. A. Aliquots from a reaction mixture similar to that described in the text but containing only 20 units of pyruvate kinase: 1, [ $\gamma\text{-}^{32}\text{P}$ ]ATP starting material; 2,  $t = 0$  min; 3,  $t = 30$  min; 4,  $t = 60$  min; 5,  $t = 90$  min; 6,  $t = 120$  min. B. Aliquots of: 1, [ $\gamma\text{-}^{32}\text{P}$ ]ATP starting material; 2, the reaction mixture before chromatography; 3, the [ $^{32}\text{P}$ ]phosphoenolpyruvate pool from the Dowex chromatography; 4, the [ $^{32}\text{P}$ ]phosphoenolpyruvate pool after treatment with 22 units/ml pyruvate kinase and 2.2 mM ADP in 0.1 M triethylamine, 0.25 M triethyl ammonium bicarbonate buffer, pH 7.8. The [ $^{32}\text{P}$ ]phosphoenolpyruvate concentration was  $9.5 \mu\text{M}$ .

The equilibrium isotope exchange process used above for the synthesis of [ $^{32}\text{P}$ ]phosphoenolpyruvate should be applicable for isotope labelling of many biochemically important compounds.

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## References

- 1 Lauppe, H.F., Rau, G. and Hengstenberg, W. (1972) FEBS Lett. 25, 357
- 2 Parra, F. (1982) Biochem. J. 205, 643–645
- 3 Mattoo, R.L. and Waygood, E.B. (1983) Anal. Biochem. 128, 245–249
- 4 Dooyewaard, G., Roossien, F.F. and Robillard, G.T. (1979) Biochemistry 18, 2990–2995
- 5 Robillard, G.T., Dooyewaard, G. and Lolkema, J. (1979) Biochemistry 18, 2984–2889
- 6 Roossien, F.F. and Robillard, G.T. (1983) Biochemistry, in the press